THE DIGESTION OF OILS BY *AMŒBA PROTEUS*.

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In a previous paper by the writers ('28) an account was given of the digestion of various oils by *Amœba dubia*. The present account deals with a series of similar experiments performed under similar conditions in which the same oils were used with another species of large free-living ameba, *Amœba proteus* (Schaeffer, '16). The procedure in this work was in every respect identical with that used with *Amœba dubia*, except that none of the oils used in this series were radiated. In the previous account as well as in this no attempt to study the rate of break-down of the various oils has been made; the sole object has been to establish the fact that a considerable variety of oils has been definitely acted on by the amebae. A study of the rate and nature of the break-down process is now in progress.

The result of this series of injections may be tabulated as follows:

Table 1.

Oil.	No. of Amebæ Injected.	No. of Amebæ Digesting Oil.	Ave. Total Vol. in μ^3 Digested per Ameba.	Ave. No. Days Injected Amebæ Lived.	Ave. No. Days Control Amebæ Lived.
1. Codliver.....	17	7	27,600	7–8	7–8
2. Cottonseed..	20	7	22,300	7.5	7.5
3. Olive.....	21	13	13,000	10.9	10.1
4. Peanut.....	21	17	12,600	8.5	6.6
5. Sperm.....	23	10	9,600	8.0	8.0
6. Linseed.....	15	8	8,800	7.5	9.0
7. Oleic.....	28	9	6,400	6.4	6.0
8. Oxfoot.....	13	8	5,300	6.0	5.4
9. Nujol.....	17	0	0,	6.0	6.0

The outstanding fact in this series of experiments with *A. proteus* is the same as in the former series with *A. dubia, i.e.*, that different oils are broken down. As can be seen by a comparison of the figures in the tables the relative digestibility of the

oils appears to differ in *A. proteus* and *A. dubia*. We wish, however, to point out that no great significance should be attached to these figures as such. They should be considered only in a broad relative aspect, although our observations lead us to the belief that distinctive differences do exist.

In any comparison of the relative volumes digested the respective volumes of the amebae must be considered. The volume of *A. proteus* was determined by the same method as that used for *A. dubia*. The average of measurements of 50 representative amebae gave an average volume of approximately 1,000,000μ^3.[1] If the ability of amebæ of these two species to break down oils is quantitatively similar it should be expected that the amounts of oil digested would be in the same ratio. The results indicate that *A. dubia* breaks down a greater volume of oil on the average than *A. proteus* although from the data given there is slight adherence to this ratio.

In the case of oleic acid and linseed oil (non-radiated in the *A. proteus* series as shown in Table 1 some digestion took place whereas none occurred in *A. dubia*. With nujol, an inert paraffin oil, as in the previous work no break-down was expected or found.

As can be seen from Table 1 there is no significant difference in most cases between the length of life of controls and experimental animals. In the case of olive and peanut oils the experimental animals lived longer on the average than the controls. In the case of linseed oil, non-radiated, the controls lived longer than the experimental animals. This effect with linseed oil may be due to the presence of traces of lead and other heavy metals which analysis showed to be present in the oil.

The entire process of breaking down of the oils in *A. proteus* is, so far as observation shows, entirely similar to the phenomenon in *A. dubia*. As in *A. dubia,* digestion did not take place in every case of injection (See Table 1). The reasons for the variation in digestion of any one oil among the individual amebæ are no doubt many; but important factors are the physiological condition of the ameba as a whole and its immediate condition from a nutritive standpoint. Temperature conditions were the same for all cases. The temperature, however, was not controlled; all the experiments being done at room temperature which varied between 68° and 74° F.

So far as our observations and data warrant it thus appears that oils of diverse types are successfully broken down by both *A. proteus* and *A. dubia* in a similar manner. Further attempts are now in progress to ascertain the precise physiological nature of this reaction in these two species of amebæ.

Some Significant Differences between *A. dubia* and *A. proteus.*

The two species of large free-living amebæ which have been used by the writers in a preceding paper ('28) and in the present work were first adequately described by Schaeffer ('16). In a recent publication (Dawson, '28) mention has been made of the fact that in long-continued mass cultures the specific differences as pointed out by Schaeffer are retained. During the course of the micro-injection studies carried on by the authors a number of fundamental differences between these two species have been disclosed.

1. *Differences in the Ability to Break Down Oils.*

As has been pointed out above the two species of amebæ show some differences in their reactions to injected oils. *A. dubia* not only did not break down the oleic acid, oxfoot and linseed oils used in this series but retained these oils for relatively short periods after injection. *A. proteus* on the other hand, in numerous cases retained and broke down these same oils.

2. *Morphological Differences.*

From the very beginning of our work difficulties in manipulative technique when working with *A. proteus* indicated that the nature of the pellicle (outermost layer) differed from that of *A. dubia.* When attempting to inject *A. proteus* using a fairly fine pipette with a slender shaft (about 2–3 μ in diameter) bending of the pipette could be seen to take place, whereas in injection of *A. dubia* such bending rarely or never occurred. If a pipette of larger diameter (about 7–8 μ) was used the pellicle of *A. proteus* in direct contact with the pipette could invariably be seen to yield or give before the pipette until the two outermost layers of the ameba almost touched each other. Such a pipette could be used to

inject *A. dubia* with comparative ease, and with a minimum amount of yielding of the pellicle before the pipette as the latter was inserted into the ameba. This would seem to indicate clearly that the pellicle of *A. proteus* is tougher than that of *A. dubia*. Our measurements have shown us that *A. dubia* is thicker in cross section than *A. proteus* (70 μ vs. 40 μ). We have found that *A. proteus* does not lend itself as easily to microinjection technique as A. dubia. The greater thinness of *A. proteus* and the toughness of its pellicle may account largely for this difficulty.

3. *Capping.*

Early in the work of injecting oil in *A. dubia* an interesting phenomenon was encountered. In many unsuccessful attempts at injection it appeared that the oil, instead of being injected into the organism, was merely brought into intimate contact with the outermost surface of the ameba. When the pipette was withdrawn the oil did not become dislodged from the surface of the ameba as might be expected but continued to remain attached to it, usually assuming roughly the form of a slightly concave hemisphere with the concave face in contact with the ameba. Whenever this occurred there was instant and typical response on the part of the organism. The protoplasm in contact with the oil extruded to form a pseudopodium, with the oil giving superficially the appearance of adhering to it at its outermost tip, and the main body of the ameba continued to flow into this pseudopodium. In about 30 seconds the entire ameba had assumed the form of the type known as *A. limax* and its endoplasmic streaming and nature of locomotion were in practically every respect identical with or markedly similar to that of an ameba of the "limax" type. In every case the ameba progressed so that the oil was pushed in advance, never dragged behind. This phenomenon the writers have termed "capping." The 'cap' might remain attached for days to the ameba and there was little or no change in either its shape or that of the modified "limax" shape of the ameba. Eventually either the cap was dislodged or the ameba died with the oil still adhering. In no case was there ever noticed any action upon the oil by the ameba which might be observed by appearance of oil in the ameba or by decrease in the size of the cap. This "capping" is

fairly easy to accomplish with *A. dubia* and when once begun invariably goes on to completion. Within one second the cap is formed and the ameba begins to assume the "limax" form immediately. With *A. proteus,* however, in no instance has a cap ever been successfully completed to remain lodged for more than a few minutes on the anterior end of the ameba as is the case in *A. dubia.* In the case of *A. proteus* only the first beginnings of the process take place; the oil when in contact with the ameba results in the formation of a very small pseudopod which protrudes in a manner very similar to that of *A. dubia* but which in a few seconds or, at most, minutes loses its contact with the oil and flows back into the main body of the ameba.

It was found that these caps as described in the preceding paragraph could be formed very frequently if a droplet of oil was forced out of the pipette but permitted to remain attached to it and then approximated to the ameba until it just came in contact and was allowed to remain thus for several seconds. If the oil was then very slightly retracted by withdrawing the pipette the capping process frequently went on to completion doing so with great speed (less than one second). For best results in obtaining caps the diameter of the droplet should not be more than approximately 50 μ. An optimum size is from 25 to 30 μ. If the droplet exceeded 50 μ in diameter there was only slight cap formation followed almost immediately by the breaking away or the separation of the oil from the ameba.

4. *Ingestion.*

During the course of these attempts it was found that if the oil was permitted to remain in contact with the ameba without retraction for several seconds, the ameba reacted by flowing around and over the oil forming a normal food cup, and, when the pipette was gently withdrawn, complete ingestion of the oil took place. After ingestion the oil was moved about in the endoplasm behaving precisely like a droplet which had been injected and was subsequently broken down in exactly the same manner as after injection. It was found that for ingestion to take place the oil had to be supported against the pipette, for if the droplet was ejected into the medium directly in the path of the ameba the

latter extruded pseudopodia toward it but ingestion failed because the streaming pseudopodium pushed the oil away from it. That this difficulty was purely a mechanical one was proved by piercing the same droplet with the pipette and holding it firmly whereupon it was ingested by the ameba. Ingestion did not take place every time a droplet of suitable size was presented to an ameba. Sometimes the ingestion reaction would be repeated several times by the same ameba unsuccessfully, followed by successful ingestion on the presentation of the oil immediately following. As is well known the previous condition of the experimental protozoon in respect to nutrition controls in large part the response in regard to further food taking. Whether this is the case in respect to this phenomenon we are unable to state at present.

The optimum size of oil droplet for ingestion comprises all sizes up to 50 μ. Very small droplets may be ingested but present technical difficulties, as they tend to adhere to the pipette and to be withdrawn from the ameba.

Contrasted with this reaction of *A. dubia* ingestion has never been accomplished with *A. proteus*. With this ameba there is only a very slight attempt at foodcup formation. *A. proteus* always moves away from the oil even when the droplet is brought into contact with the animal and held so for several seconds.

The breakdown of ingested droplets in *A. dubia* takes place so far as observation and quantitative data show in precisely the same way as with injected droplets. So far, ingestion experiments have been carried on with five oils, viz., nujol, olive, codliver, sperm and cottonseed. *A. dubia* ingested all of these oils in the same way. In no case of ingestion of oil droplets was a vacuole ever formed about the oil. Likewise no vacuole was ever formed about any injected oil drop.

5. *Comparative Length of Life of Controls.*

The behavior of these two species of ameba when placed in distilled water as controls was markedly different from the standpoint of length of survival. *A. dubia* lived on the average from 4 to 5 days under these conditions at room temperature. None survived beyond six days. The average length of life for *A. proteus* under the same conditions was from 7 to 8 days with cer-

tain series living up to 11 days, and with numerous instances of individuals living for 18 days. This indicates a greater degree of hardiness on the part of *A. proteus* which is fully substantiated by experience in the mass culture of both these organisms.

Summary.

1. *A. proteus* successfully breaks down, after micro-injection, the following oils: codliver, cottonseed, olive, peanut, sperm, linseed, oleic, oxfoot.

2. A number of significant differences have been found between *A. dubia* and *A. proteus*.

a. In their respective ability to break down oils.

b. Morphological differences as revealed by injection, measurement of volume and nature of pellicle.

c. Under certain conditions *A. dubia* undergoes the phenomenon of ' capping ' with oil. No permanent " capping " ever takes place with *A. proteus*.

d. *A dubia* will under suitable conditions ingest oil. *A. proteus* under similar conditions never ingests oil.

e. *A. proteus* has the ability to live longer under similar adverse conditions than *A. dubia*.

LITERATURE CITED.

Dawson, J. A.

'28 The Culture of Large Free-living Amebæ. Amer. Nat., Vol. 62, 453–466.

Dawson, J. A., and Morris Belkin.

'28 The Digestion of Oils by *Amœba dubia*. Proc. Soc. Exp. Biol. and Med., Vol. 25, 790–793.

Schaeffer, A. A.

'16 Notes on the Specific and Other Characters of *Amœba proteus* Pallas (Leidy), *A. discoides* spec. nov., and *A. dubia* spec. nov. Arch. f. Protistenk., Bd. 37, 204–228.

[1] Due to error the volume of *A. dubia* was given as 500,000 μ^3. This should be 2,500,000 μ^3. Thus the percentages given in Table 1, column 4, in our previous paper should be multiplied by 0.2.